THE POTENTIAL FOR THE MARINE BIOTECHNOLOGY INDUSTRY

Shirley A. Pomponi
Harbor Branch Oceanographic Institution, Florida

Introduction

The marine environment is a rich source of both biological and chemical diversity. This diversity has been the source of unique chemical compounds with the potential for industrial development as pharmaceuticals, cosmetics, nutritional supplements, molecular probes, enzymes, fine chemicals, and agrichemicals. Each of these classes of marine bioproducts has a potential multi-billion dollar market value (BioScience, 1996). Thousands of unique chemical compounds have been identified from a relatively small number of the ocean's biological and chemical diversity (Ireland et al, 1993). The oceans represent a virtually untapped resource for discovery of even more novel compounds with useful activity.

There are several marine-derived products currently on the market (Table 1). Although this discussion will focus on the current status and future potential of marine biotechnology related to the discovery, development, and sustainable use of marine-derived compounds with biomedical applications, the needs, approaches, and opportunities apply equally to other marine bioproducts. The challenge facing the marine biotechnology industry in the next millenium is to:

- identify new sources of marine bioproducts;
- develop novel screening technologies;
- provide a sustainable source of supply; and
- optimize production and recovery of the bioproducts.

Identification of New Sources of Marine Bioproducts

Marine bioproducts have, to date, been derived from relatively shallow-water organisms using routine methods, such as scuba diving. Evaluation of the pharmaceutical, cosmetic, nutritional, and chemical potential of products derived from deep water organisms has been limited, although at least one compound—discodermolide (Gunasekera et al, 1990; ter Haar et al., 1996), derived from a deep water sponge—has been recently licensed by Harbor Branch Oceanographic Institution to Novartis Pharma AG, and is in advanced preclinical trials for treatment of cancer.

Federal agency support (e.g., NSF, NOAA, ONR, NIH) for deep ocean exploration for biotechnology is limited, at best. Manned and unmanned submersibles are woefully underfunded and restricted to a few systems. The trend toward development of remote platforms for understanding the oceans and atmosphere has had little application relative to marine biodiversity—and the potential of this diversity to yield useful products. Despite the trend toward remotely operated systems, there is still a need for manned submersible programs to study and sample biodiversity in the deep oceans. Although some submersible systems are equipped with specialized tools and chambers that allow samples to be maintained under ambient conditions, i.e., high pressure and, low temperature, there is still a need for the development of versatile bioreactors that can be deployed and operated in extreme environments

Table 1. Some Examples of Commercially Available Marine Bioproducts

Product	Application	Original Source
Ara-A	antiviral drug	marine sponge, Cryptotethya crypta
Ara-C	anticancer drug	marine sponge, Cryptotethya crypta
okadaic acid	molecular probe: phosphatase inhibitor	dinoflagellate
manoalide	molecular probe: phospholipase A2 inhibitor	marine sponge, Luffariella variabilis
Vent™ DNA polymerase	polymerase chain reaction enzymes	deep-sea hydrothermal vent bacterium
Formulaid® (Martek Biosciences, Columbia, MD)	fatty acids used as additive in infant formula nutritional supplement	marine microalga
Aequorin	bioluminescent calcium indicator	bioluminescent jellyfish, Aequora victoria
Green Fluorescent Protein (GFP)	reporter gene	bioluminescent jellyfish, Aequora victoria
phycoerythrin	conjugated antibodies used in ELISAs and flow cytometry	red algae
Resilience® (Estée Lauder)	marine extract additive in skin creams	Caribbean gorgonian, Pseudopterogorgia elisabethae

(e.g., hypersaline, vent, anoxic, and deep-sea habitats). Such bioreactors could be used for collection, at-sea maintenance, and evaluation of novel macroorganisms and microorganisms so that their metabolites can be evaluated under physiological conditions that are as similar as possible to ambient conditions.

Another approach to the identification of new products is the incorporation of miniaturized biosensors into both collecting tools and bioreactors for rapid, in situ analysis of both wild and cultivated marine organisms for target molecules. A number of miniaturized biosensors and probes to study human disease processes are in development. Adaptation of these for in situ evaluation of marine-derived products would be an interesting bioengineering challenge. Potential applications are the identification of new or previously untested species, as well as analysis of gene expression that may be specific to a particular disease or therapeutic area.

With the enormous potential for discovery, development, and marketing of novel marine bioproducts comes the obligation to develop methods by which these products can be supplied in a way that will not disrupt the ecosystem or deplete the resource.

Development of Novel Screening Technologies

The biological evaluation of marine-derived extracts and pure compounds for pharmaceutical development has been based on assays developed for the high-throughput screening of large libraries of synthetic compounds. They measure a number of end-points, such as activation or inhibition of enzymes or receptors involved in human disease processes, inhibition of growth of human pathogenic microorganisms, and toxicity against human cancer cells (Ireland et al, 1993; McConnell et al, 1994; Munro et al, 1994). None of the assays used in major pharmaceutical drug discovery programs takes into account the role of marine-derived compounds in nature, i.e., the in situ biochemical functions of both primary and secondary metabolites, and how those functions may be applied to the discovery of new drugs and probes to study human disease processes. Marine organisms as model systems offer the potential to understand and develop treatments for disease based on the normal physiological role of their secondary metabolites. For example, the mechanisms of action Conus toxins are well-known (Hopkins, et al, 1995; Shon et al, 1997), and are currently being applied to the development of new classes of drugs. Development of in situ biosensors would enhance our ability to probe the expression of secondary metabolites in response to various stimuli, lead to a better understanding of the role of the secondary metabolites in nature, and perhaps provide clues to the potential biomedical utility of these compunds

Sustainable Use of Marine Resources

With the enormous potential for discovery, development, and marketing of novel marine bioproducts comes the obligation to develop methods by which these products can be supplied in a way that will not disrupt the ecosystem or deplete the resource. Supply of most marine-derived compounds is a major limiting factor for further pharmaceutical development. Often, the metabolite occurs in trace amounts in the organism, and a steady source of supply from wild harvest cannot provide enough of the target compound for preclinical studies. In general, the natural abundance of the source organisms will not support production based on wild harvest.

Some options for sustainable use of marine resources are chemical synthesis, controlled harvesting, aquaculture of the source organism, in vitro production through cell culture of the macroorganism or microorganism source, and transgenic production. Each of these options has its advantages and limitations. Not all methods will be applicable to the supply of every marine bioproduct, and most of the biological supply methods are still in development. The approach to be used will be based on a number of factors:

- Complexity of the molecule: Can it be synthesized using an industrially feasible process? Synthetic processes have been published for many marine bioproducts in development as pharmaceuticals. Unfortunately, most of these are multi-step processes that are not amenable to economic, industrial-scale synthesis.

- Abundance of the organism in nature: What do we know about the impact of collections on the habitat or species populations? Prior to large-scale wild harvest of an organism for recovery of

a bioproduct, harvesting feasibility studies should be conducted. These should define factors such as the standing stock of the organism, its growth rate and the factors that affect growth, and the harvesting and post-harvesting recovery of the target organism. These impact data could then be used not only to assess the potential of supply from wild harvest, but also to develop models for aquaculture and / or in vitro production. Unfortunately, this is rarely done.

- Source of the compound: Is it microbially produced? A significant number of marine bioproducts with pharmaceutical potential have been identified from heterotrophic marine microorganisms isolated from coastal sediments (Fenical, 1993; Davidson, 1995; Kobayashi and Ishibashi, 1993). In addition, some marine bioproducts originally isolated from macroorganisms, such as sponges, have been subsequently discovered to be localized in microbial associates (e.g., Bewley et al, 1996). If these symbiotic microorganisms can be isolated and cultured, optimization of production in marine microbial bioreactors may lead to an industrially feasible supply option. If the source of the compound is the macroorganism itself, development of in vitro production methods could provide bulk supply of the compound. Research in progress in our laboratory focuses on establishing cell lines of bioactive marine invertebrates that can be used as models to study in vitro production of bioactive metabolites and the factors which control expression of production (Pomponi et al, 1997, 1998). This could ultimately lead to in vitro production of marine bioproducts. More importantly, an understanding of the cellular and molecular processes that control production of these metabolites could be used to enhance upstream processing / culture optimization and to stimulate production of "unnatural" natural products—i.e., chemicals that the organism would not produce under normal conditions, but which may be more potent than the "natural" product.

- In situ growth conditions: Is aquaculture an option for deep-water organisms? Both in-the-sea and land-based aquaculture methods have been developed for production of bioproducts from shallow-water organisms. CalBioMarine Technologies (Carlsbad, CA) has successfully aquacultured the bryozoan, Bugula neritina, and Ecteinascidia turbinata, the ascidian from which the antitumor compound, ecteinascidin 743, has been isolated (Wright et al, 1990; Rinehart et al, 1990). These are both common, shallow-water organisms for which reproduction and growth have been studied, but the factors controlling production of the compounds are not yet completely known. The New Zealand deepwater sponge, Lissodendoryx sp., is the source of the antitumor compounds, halichondrins. The sponge occurs at 85-105 meters, but has been cultured successfully from cuttings on lantern arrays in shallower water, maintaining production of the bioactive halichondrins (Battershill et al, 1998). Current efforts are directed toward modification of metabolite production by altering the microenvironment (Battershill, personal communication). This indicates that aquaculture of some deep water sponges is feasible; however, species from deeper water may have more critical growth requirements, such as high pressure and low temperature.Although in-the-sea aquaculture is a cost-effective method of production, it may not afford the opportunity for over-expression of production of the compounds or for complete control of environmental parameters. Development of closed-system bioreactors for the culture of both shallow water and deep water organisms is a particularly challenging opportunity for marine bioprocess engineers.

- Biosynthetic pathway: Is genetic engineering realistic for the compound? If the biosynthesis of the target compound is understood, it may be possible to identify, isolate, clone, and express in a heterologous host the genes responsible for production of the metabolite. In many cases, of course, biosynthesis of the product is not known, or it is a multi-step process involving several enzymatic reactions. For these cases, transgenic production is not a trivial process. Alternatively, chemoenzymatic synthesis, by which marine bioproducts are synthesized in cell-free, enzyme-based systems, offers a complementary technique to in vitro and transgenic production methods for marine bioproducts (Kerr et al, 1996 a, b).

Optimization of Production

Perhaps the area in which marine biotechnology in general, and marine bioprocess engineering in particular, has the greatest potential is in the design and optimization of bioreactors for marine metabolite production. A variety of bioreactor designs have been implemented, with varying degrees of success. The opportunity to produce new, bioactive structural analogs of known compounds via manipulation of

culture conditions presents marine biotechnologists with a unique challenge for new bioproduct discovery. Innovations in media development (chemical engineering), bioreactor design (bioprocess engineering), and transgenic production (molecular engineering), coupled with efficient downstream processing and product recovery, will be necessary to meet the needs of both discovery and bulk production of novel marine bioproducts.

In summary, the marine biotechnology industry faces a unique challenge for the millenium: Inventing a new generation of tools and processes that will enable a greater understanding of the ocean and its resources and lead to the discovery of new bioproducts for the future, and designing methods for the sustainable development of these unique bioproducts.

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